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Catabolism of histamine in the isolated glomeruli and tubules of the rat kidney

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Catabolism of histamine in the isolated glomeruli and tubules of the rat kidney. Histamine alters renal hemodynamics including the glomerular microcirculation, and histamine receptors are present in rat glomeruli. We have recently shown that isolated rat glomeruli, but not cortical tubules, incubated with the histamine substrate L-histidine synthesize histamine. This study explores the catabolic pathways of histamine in isolated glomeruli and cortical tubules of the rat kidney. Glomeruli and cortical tubules were incubated with radiolabelled histamine, and the products were separated on thin layer chromatography (TLC). Glomeruli predominantly catabolized histamine to acid metabolites of the diamine oxidase (histaminase) pathway, imidazole acetic acid and ribosylimidazole acetic acid, and to a lesser extent to the inactive methylation product, Nτ-methylhistamine (7.5% vs. 2.5%). Tubules on the other hand catabolized histamine to Nτ-methylhistamine and to a lesser degree to acid metabolites (7.6% vs. 2.3%). The methyl donor S-Adenosyl-methionine (SAM) (10^{-4}M) markedly enhanced the production of Nτ-methylhistamine in both glomeruli and tubules (Δ + 600%) but had no effect on the production of acid metabolites. In the presence of equimolar concentrations of SAM, tubules continued to methylate histamine to a greater extent than glomeruli (46.0% vs. 18%). In both glomeruli and tubules, the diamine oxidase inhibitor, amino-guanidine, abolished the production of acid metabolites while amodiaquine and pyrilamine, inhibitors of the methylation pathway, markedly reduced the production of Nτ-methylhistamine. In addition, in the presence of SAM, tubules catabolized nonlabelled histamine to a greater extent than glomeruli. These studies show that tubules have a greater capacity than glomeruli to degrade histamine and that histamine is differentially catabolized in these segments. A major pathway of histamine catabolism in glomeruli results in the formation of biologically active products.

Catabolisme de l'histamine dans des glomérules et des tubules isolés de rein de rat. L'histamine altère l'hémodynamique rénale notamment la microcirculation glomérulaire, et des récepteurs de l'histamine sont présents dans les glomérules de rat. Nous avons récemment montré que des glomérules isolés de rat, mains non des tubules corticaux, incubés avec de la L-histidine, le substrat de l'histamine, synthétisent de l'histamine. Cette étude explore les voies cataboliques de l'histamine dans des glomérules et des tubules corticaux isolés de reins de rat. Les glomérules et les tubules corticaux ont été incubés avec de l'histamine radiomarquée, et les produits ont été séparés par chromatographie en couche mince (TLC). Les glomérules catabolisaient préférentiellement l'histamine en des métabolites acides de la voie de la diamine oxidase (histaminase), l'acide imidazole acétique et l'acide ribosylimidazole acétique, et à un moindre degré en produit de méthylation inactif, le Nτ-méthylhistamine. Les tubules, quant à eux, catabolisaient l'histamine en Nτ-méthylhistamine et à un moindre degré en métabolites acides (7.6% contre 2.3%). La S-Adénosyl-méthionine (SAM) (10^{-4}M), un donneur de méthyle, a stimulé de façon marquée la production de Nτ-méthylhistamine dans les glomérules et les tubules (Δ + 600%) mais n'avait pas d'effet sur la production des métabolites acides. En présence de concentrations équimolaires de SAM, les tubules continuaient de méthyler l'histamine de façon plus importante que les glomérules (46.0% contre 18%). Dans les glomérules comme dans les tubules, un inhibiteur de la diamine oxidase, l'amino-guanidine, a supprimé la production de métabolites acides, alors que l'amodiaquine et la pyrilamine, des inhibiteurs de la voie de méthylation, ont réduit considérablement la production de Nτ-méthylhistamine. En outre, en présence de SAM, les tubules catabolisaient l'histamine non marquée de façon plus importante que les glomérules. Ces études montrent que les tubules ont une plus forte capacité que les glomérules à dégrader l'histamine, et que l'histamine est catabolisée de façon différente dans ces segments. Une voie majeure du catabolisme de l'histamine dans les glomérules aboutit à la formation de produits biologiquement actifs.

There is increasing evidence that the vasoactive amine histamine may play an important role in modulating renal function under physiological conditions or in the course of renal injury [1–10]. In this context, histamine has been shown to dilate the renal circulation and to increase salt and water excretion [1–3], to decrease the ultrafiltration coefficient [3], and to increase renin release [4]. In addition, studies utilizing the systemic administration of antihistamines [5–8] strongly suggest a role for histamine in mediating the renal injury in some [5, 6] but not all [7, 8] forms of experimental glomerulonephritis. Very few studies have examined the intrarenal metabolism of histamine [9–11]. Isolated rat glomeruli have a high activity of the specific histidine decarboxylase [11]. We and others recently demonstrated that isolated rat glomeruli, but not cortical tubules, are capable of synthesizing histamine when incubated with the substrate L-histidine [10] and that histamine acting via an H_2 receptor specifically alters glomerular cyclic nucleotide levels [12, 13]. These observations suggest a biological role of the amine in this nephron structure. The rates of synthesis and catabolism of histamine are among the major factors that influence the dynamics of histamine metabolism and availability in tissues [14]. The catabolism of histamine proceeds via two major enzymatic pathways [14–16]: *(1)* oxidative deamination by histaminase (diamine oxidase) to imidazole acetic acid (IMAA) which could be conjugated to form ribosyl imidazole acetic acid; *(2)* methylation of the imidazole ring of histamine by histamine methyl transferase to Nτ-methylhistamine (Nτ-MH) which may be subsequently oxidized to Nτ-methylimidazole acetic acid (Nτ-MIAA). The ability of whole kidney tissue to catabolize histamine in vitro and in vivo has been recognized

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for a long time [15, 16]. However, the precise localization and the contribution of each pathway to histamine catabolism within the kidney are not known. It has recently become evident that a variety of histamine metabolites, which were formerly treated as inactive byproducts, possess significant biologic activity [14, 17, 18]. Our studies were designed to examine the catabolic pathways of histamine in glomeruli and a mixed population of cortical tubules.

Methods

L-histamine[Ring-2-^{14}C] dihydrochloride 59.7 mCi/mmole, and S-adenosyl-L-methionine-[^{14}C-methyl] 57.9 mCi/mmole were purchased from Amersham Corp., Arlington Heights, Illinois. The ^{14}C-labelled histamine was purified on cellulose plates as described [19]. [(β-side chain label)-^{3}H] histamine was prepared from [(β-side chain label)-^{3}H] L-histidine 10 Ci/mmole (New England Nuclear Corp., Boston, Massachusetts) as described previously [10]. Non-labelled S-Adenosylmethionine was obtained from Boehringer, Manheim Biochemicals, Indianapolis, Indiana. Non-labelled histamine dihydrochloride, L-histidine, imidazole acetic acid, and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Missouri). 1-Methyl-4-(2-aminoethyl) imidazole hydrochloride (Nτ-methylhistamine) was purchased from Calbiochemical Corp. (San Diego, California). Pyrilamine maleate was a product of K and K Laboratories, Inc., (Plainview, New York). Aminoguanidine sulphate was purchased from Eastman Organic Chemicals, Eastman Laboratory and Speciality Chemicals, Eastman Kodak Co., Rochester, New York. Amodiaquine was kindly supplied by Dr. S. A. Fusari, Park, Davis and Co. (Detroit, Michigan).

Isolation of glomeruli and tubules. Kidney tissue used for preparation of glomeruli and tubules was obtained from male Sprague-Dawley rats weighing 200 to 250 g, maintained on a standard diet, (Purina Laboratory Rat Chow, Ralston Purina Co., St. Louis, Missouri), and having free access to tap water. Rats were anesthesized and the kidneys were perfused in situ with 60 to 80 ml of modified Krebs Ringer Phosphate Buffer (KRB) until surfaces were completely blanched. The kidneys were then quickly excised, decapsulated and placed in an ice-cold KRB. Glomeruli and tubules were prepared from renal cortical tissue by a combination of sieving and differential centrifugation as described in our previous reports [10, 13]. The purity of each glomerular suspension (95 to 98%) was evaluated by light microscopic examination and counting of glomeruli [10, 13]. Glomeruli or tubules were then resuspended in 0.1 M sodium phosphate buffer containing 2.7 mM KCl, 0.8 M $CaCl_2$, and 0.1% glucose (incubation buffer) so that 8 to 12 mg of glomeruli or tubules were contained in 100 μl.

Catabolism of histamine in isolated glomeruli and tubules. Freshly prepared glomeruli and tubules suspended in incubation buffer were distributed into 1.5 ml microcentrifuge tubes (Eppendorf) kept on crushed ice at 0°C. Inhibitors, labelled histamine, and the methyl donor S-Adenosylmethionine (SAM) were added in that order when appropriate. The final incubation volume was 200 μl. Tubes were mixed thoroughly and incubated for 120 min, or as specified in **Results**, at 37°C in a metabolic, shaking water-bath. In addition, the tubes were also shaken manually over 30 min during the incubation. In each experiment, blank incubations were run using glomerular and tubular suspensions which were heated in a boiling water bath for 10 min before the addition of the various agents. At the end of the incubation, the reaction was stopped by the addition of 10 μl of a solution containing amodiaquine and aminoguanidine (each in a concentration of 2 2×10^{-4}M); in addition, 10 μl solution containing cold histamine, imidazole acetic acid, and methylhistamine, each in a concentration of 0.75 mg/ml, were added to all tubes as a carrier and to facilitate the extraction and identification of labelled histamine and metabolites. Tubes containing glomerular and tubular suspensions were then frozen and stored at −20°C.

Extraction of histamine and metabolites. The extraction method of Code et al [20] was carried out in all the experiments with radiolabelled histamine. In preliminary experiments, we found that this extraction step is necessary for clear and reproducible separation of histamine and its metabolites. Glomerular and tubular suspensions in microcentrifuge tubes were frozen and thawed in an ethanol/dry ice bath (four times) to insure release of histamine and metabolites. A 10-μl aliquot was taken for the determination of total radioactivity. Trichloroacetic acid (200 μl of 20% TCA) was then added to precipitate the proteins. After 30 min in the cold, proteins were precipitated by centrifugation at $\times 12{,}800g$ for 30 min in a microcentrifuge (Eppendorf). The supernate was transferred to clean tubes and the pellet was resuspended in 100 μl of 20% TCA and centrifuged again. The supernates were combined. TCA was removed from the supernate by three extractions with three volumes of water-saturated ether. The supernatant solution was then dried using a centrifugal vacuum evaporator (Savant Instruments, Hicksville, New York). The dried material was taken up in 0.5 ml of acidified methanol and centrifuged; the supernate was transferred to fresh tubes and evaporated using the centrifugal vacuum evaporator. The dried residue was dissolved in a 50-μl volume of absolute methanol to provide about 1500 dpm/μl. A 10-μl aliquot of the reconstituted absolute methanol was counted to determine the recovery. Of the radioactivity present in the original homogenate 76.0% ± 4.5 (SEM) was recovered in the final alcoholic extract. Aliquots of the methanolic extracts measuring 10 μl were then applied to 1.0-cm regions on 20 × 20 cm silica gel chromatogram plastic sheets without fluorescent markers (EM, E. Merck, Darmstadt, Germany). The dried plates were activated for 60 min at 110°C prior to use. The chromatogram sheets were marked 1 inch from the bottom and 1.0 cm from each side into 1-cm sample application regions, each separated from one another by 1.5 cm. The sheets were chromatographed in an ascending direction 16 cm in glass chromatogram chambers using methanol: chloroform: ammonium hydroxide 12:7:1 as solvents [21, 23]. Ten microliters of a standard solution of histamine, imidazole acetic acid, and methylhistamine (each in a concentration of 0.1 mg/ml) were also placed on each plate and co-chromatographed. After drying the plates thoroughly, histamine and methylhistamine were identified by spraying with ninhydrin and imidazole acetic acid by spraying with sulphanilic acid reagent [20, 23]. In this system, histamine and its major metabolites were well separated and could be measured accurately. As soon as the plates were developed and stained, the spots representing histamine and its two major metabolites were outlined, and the chromatographic strip was divided into 1- to 1.5-cm wide spots. The areas representing each spot were cut by scissors and

placed into separate vials. The radioactivity was estimated by liquid scintillation measurement of radioactivity in a cut section of the chromatogram suspended in 15 ml of scintillation fluid (Aquasol, New England Nuclear Corp., Arlington Heights, Illinois).

Studies with nonlabelled histamine. The catabolism of histamine in glomeruli and tubules was also examined utilizing nonlabelled histamine as substrate, and histamine levels were then determined by radioenzymatic assay [24]. The incubation conditions were similar to those with labelled histamine with minor modifications. The glomerular and tubular suspensions were incubated with various concentrations of nonlabelled histamine in the absence and in the presence of SAM. Control tubes were incubated with boiled suspensions of glomeruli and tubules. SAM was also added to the control tubes of the incubations carried out in the presence of SAM. At the end of the incubation, all tubes were immersed in boiling water for 10 min to terminate the reaction and destroy SAM [24]. The tubes were then frozen and stored at −20°C before histamine determination. For determination of histamine, the tubes were then frozen and thawed three times and centrifuged at ×12,800*g* for 30 min. The supernate was separated and adjusted to pH 7.9. Proteins in the precipitate were assayed by the method of Lowry et al [25]. Histamine was detemined at 50-μl aliquots of the supernatants by a double isotope radioenzymatic assay [10, 24]. Samples containing high concentrations of histamine were diluted with 0.1 M Na phosphate buffer pH 7.9 prior to assay.

Statistical Analysis. Differences between glomeruli and tubules were analyzed using unpaired two-tailed Student's *t* test, and paired one-tailed *t* test for changes within glomeruli or tubules.

Results

Glomeruli and cortical tubules incubated under the same conditions catabolized ^{14}C-labelled histamine to major products that could be separated on silica gel TLC. Figure 1 shows the distribution of radioactivity in a representative experiment in the absence and presence of methyl donor SAM. A ^{14}C-peak occurred at the position occupied by IMAA. This peak was higher in glomeruli than in tubules, 7.45% vs. 2.40%, and was little affected by the addition of SAM to the incubation, 7.60% vs. 2.25% of total radioactivity, respectively. In this system however, imidazole acetic acid riboside, the conjugated metabolite of IMAA, and Nτ-methyl imidazole acetic acid, the deaminated product of methylhistamine, cannot be separated from IMAA [21]. That Nτ-MIAA does not contribute significantly to the radioactivity in this peak is suggested by the absence of change in counts over this spot both by the addition of SAM, which enhances the radioactivity of Nτ-MH spot by several folds, and by the inclusion of specific methylation inhibitors which abolish the production of Nτ-MH. Furthermore, we observed no change in counts when incubations were carried out in the presence of tranylcypramine, a monoaminoxidase inhibitor (data not shown). This peak was therefore taken as representative of the acidic metabolites of the diamine oxidase pathway: imidazole acetic acid and ribosyl imidazole acetic acid (IMAA + R). The identity of this peak as representative of acid metabolites of the diamine oxidase pathway is confirmed by the studies utilizing the specific diamine oxidase inhibitor aminoguanidine (Table 1). Another ^{14}C peak occurred

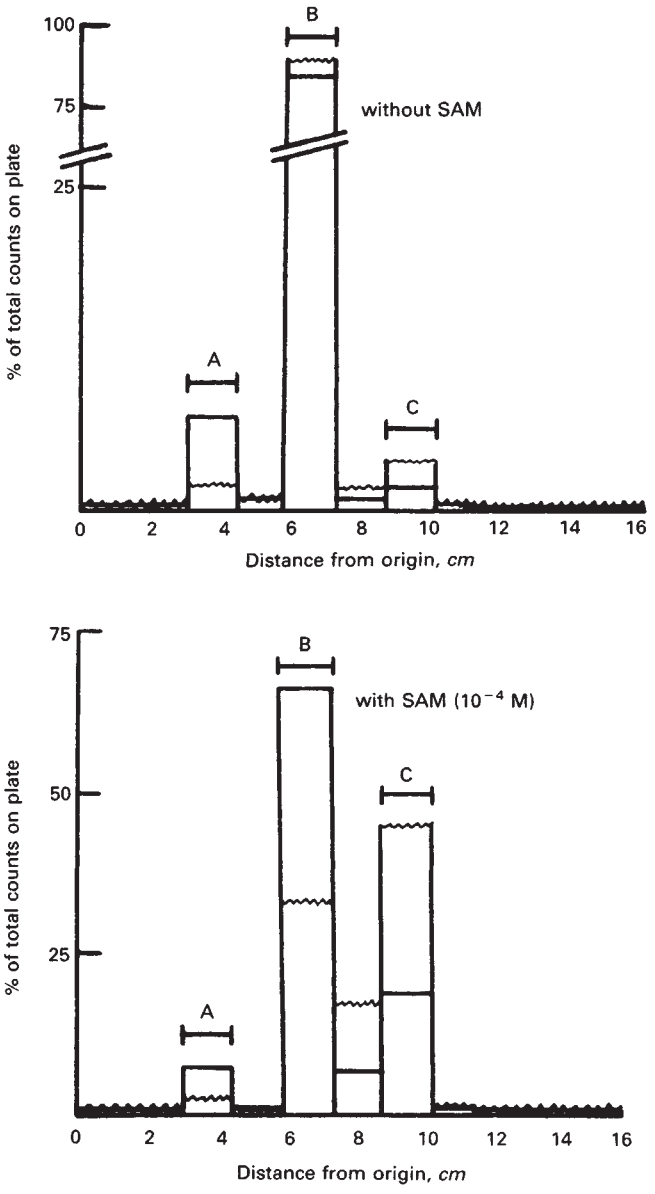

Fig. 1. *TLC of ^{14}C-histamine and its products in glomeruli* (solid bars) *and tubules* (hashed bars) *incubated in the absence* (upper panel) *and the presence* (lower panel) *of 10^{-4}M S-Adenosylmethionine* (SAM). Note the difference in the scales. The positions of reference compounds are indicated by the letters: A, IMAA + R (imidazole acetic acid and ribosylamidazole acetic acid); B, H (histamine); C, N -MH (Nτ-methylhistamine). Values shown in this and in all subsequent experiments are after subtracting the blank (incubation and TLC performed under identical conditions but in the presence of boiled tissue). Incubations were carried out in the presence of 5×10^{-6}M ^{14}C-histamine for 120 min. Total radioactivity recovered averaged 16,580 dpm. Protein content in glomeruli averaged 485 μg and in tubules 467 μg per incubation sample.

at the position occupied by ring-methylated histamine (Nτ-MH). This peak was higher in tubules than in glomeruli both in the absence and presence of SAM, 4.5% vs. 2.38% and 45.5% vs. 18.2% of the total recovered radioactivity, respectively. The identity of this product as that of the methylation pathway was confirmed by the studies utilizing the methylation inhibitors

Table 1. Effect of inhibitors on the catabolism of histamine in isolated glomeruli and tubules[a]

			Amodiaquine		Aminoguanidine	
		Control	10^{-7} M	10^{-6} M	10^{-7} M	10^{-6} M
Effect of amodiaquine and aminoguanidine						
Nτ-MH	Glom	324.0[b] ± 46.0	310.8 ± 52.2	143.0 ± 28.5	339.8 ± 20.8	277.0 ± 27.8
	Tub	595.3 ± 88.9	379.0 ± 17.6	198.0[c] ± 55.8	468.6[d] ± 31.3	549.7[d] ± 49.8
IMAA + R	Glom	104.3 ± 17.6	86.5 ± 12.2	89.9 ± 21.4	11.4[c] ± 3.7	4.1[c] ± 2.4
	Tub	17.5[d] ± 7.6	16.4[d] ± 7.0	14.2[d] ± 6.4	2.8[c] ± 1.0	4.1 ± 3.3
Effect of pyrilamine						
			Pyrilamine			
			10^{-5} M	10^{-4} M		
Nτ-MH	Glom	295.3 ± 21.5	276.0 ± 40.0	106.6[c] ± 27.6		
	Tub	562.0[d] ± 42.6	431.7 ± 49.8	207.0[c] ± 50.9		
IMAA + R	Glom	73.3 ± 22.3	70.3 ± 11.9	65.5 ± 4.7		
	Tub	16.5[d] ± 5.8	23.4[d] ± 2.1	38.1 ± 6.1		

Abbreviations: Nτ-MH, Nτ-methylhistamine; IMAA + R, imidazole acetic acid and ribosyl imidazole acetic acid.

[a] Incubations were carried out in the presence of 5×10^{-6}M ^{14}C-labelled histamine and 10^{-5}M S-Adenosylmethionine for 120 min. Labelled histamine and metabolites were separated on TLC. For details, see **Methods.**

[b] Mean ± SEM of values from three experiments (each determined in duplicate or triplicate incubation samples); data are expressed as pmoles/mg protein.

[c] Significantly different from corresponding control levels (without inhibitors) at ($P < 0.05$ or higher degree of significance; paired t test).

[d] Levels in tubules significantly different from corresponding levels in glomeruli ($P < 0.05$ or higher degree of significance; unpaired t test).

(amodiaquine and pyrilamine) (Table 1). Another less prominent peak of radioactivity migrated between histamine and Nτ-methylhistamine. The pattern of radioactivity over this spot, both in the presence and absence of SAM, resembled that produced by Nτ-methylhistamine in glomeruli and tubules but was of a much lower magnitude. Although this peak probably represents a methylation metabolite [21], we have not attempted further identification of this product. The percent counts over all other remaining spots were less than 1% of the total radioactivity.

Figure 2**A** and **B** shows that there was a progressive increase in product formation for at least 150 min of incubation in the absence and presence of SAM. When the glomerular and tubular protein concentration was progressively increased (Fig. 3**A** and **B**), there was a corresponding increase in the production of both metabolites, up to at least 500 μg tissue protein. Subsequent incubations were therefore carried out in glomerular and tubular suspensions containing 300 to 500 μg protein (approximate wet weight of 6 to 12 mg) per incubation sample for 120 min.

The addition of increasing concentrations of SAM to the incubation medium (Fig. 4) enhanced the production of Nτ-methylhistamine in both glomeruli and tubules but had no effect on the production of (IMAA + R). The production of Nτ-MH by tubules continued to be much higher than that in glomeruli at all tested concentrations of SAM.

Figure 5 shows the effect of varying the concentration of radiolabelled histamine in the incubation medium on the production of both products. There was a progressive increase in the production of (IMAA + R) and Nτ-MH in both glomeruli and tubules. At all concentrations of histamine tested, the production of Nτ-MH in tubules exceeded that in glomeruli while the production of (IMAA + R) in glomeruli exceeded that in tubules.

Figure 6 shows the data from four separate experiments performed in the absence and presence of saturating concentrations of SAM (10^{-4}M). Both in the absence and presence of the methyl donor SAM, tubules catabolize histamine to methylhistamine to a much greater extent than glomeruli. The addition of SAM enhanced the methylation pathway by more than tenfold in both glomeruli and tubules. However, glomeruli predominantly catabolized histamine to (IMAA + R) and the amount of (IMAA + R) produced was not affected by the addition of SAM. Tubules catabolized histamine to (IMAA + R) to a much lesser degree. To eliminate the possibility that a higher uptake of SAM or radiolabelled histamine by tubules as compared to glomeruli was a cause of the differential catabolism of histamine, glomeruli and tubules were permeabilized by repeated freezing and thawing (three times) prior to the incubation with radiolabelled histamine. Production of Nτ-MH in tubules and glomeruli after this procedure averaged 940.0 ± 42.0 vs. 390.0 ± 26.0, respectively (pmoles/mg protein; mean ± SEM of three incubation samples).

Table 1 shows that 10^{-6}M amodiaquine, an inhibitor of the methylation pathway, [14, 15] inhibited the production of Nτ-MH in both glomeruli and tubules but had no effect on the production of IMAA + R. Conversely, 10^{-7}M and 10^{-6}M aminoguanidine, a specific inhibitor of the diamine oxidase pathway, [14, 15] abolished the production of IMAA + R but had no effect on the production of Nτ-MH. Pyrilamine, an H1 receptor antagonist, and a known inhibitor of the methylation pathway [14], also inhibited the production of Nτ-MH but had no effect on the production of IMAA + R.

Finally, to verify the magnitude of histamine catabolism observed in the TLC studies, experiments were carried out with nonlabelled histamine, and histamine levels were determined by radioenzymatic assay. The percent recovery of histamine (50%) measured by the radioenzymatic assay in the samples incubated with SAM was similar to that incubated without SAM and confirms previous observations that SAM is totally destroyed by boiling [24]. In the absence of added SAM, and at both concentrations of exogenous histamine (0.5×10^{-6}M and $5 \times$

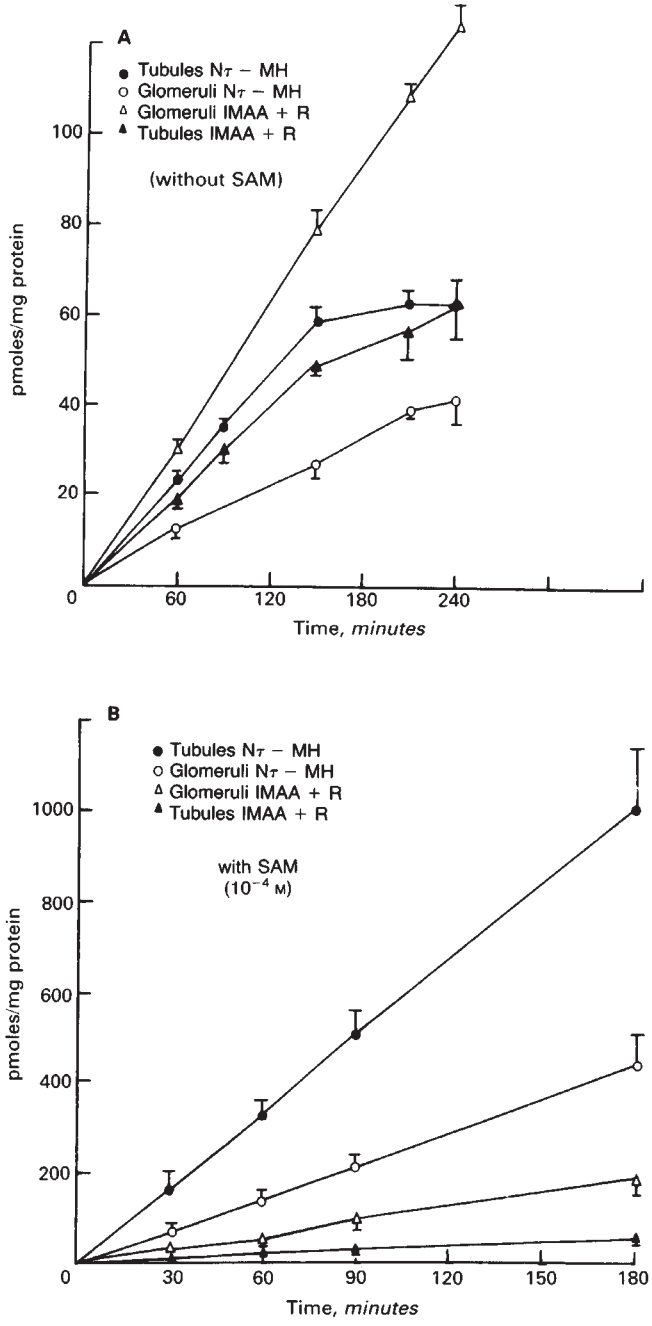

Fig. 2. *Time course of product formation in isolated glomeruli and tubules.* Incubations were carried out in the presence of 5×10^{-6}M ^{14}C-histamine for 120 min in the absence (**A**) and presence (**B**) of 10^{-4}M S-Adenosylmethionine (SAM). Labelled histamine and products were separated on thin layer chromatography. Note the difference in the scales. Each *point* represents the mean ± SEM of four determinations (two incubation samples aliquoted in duplicates).

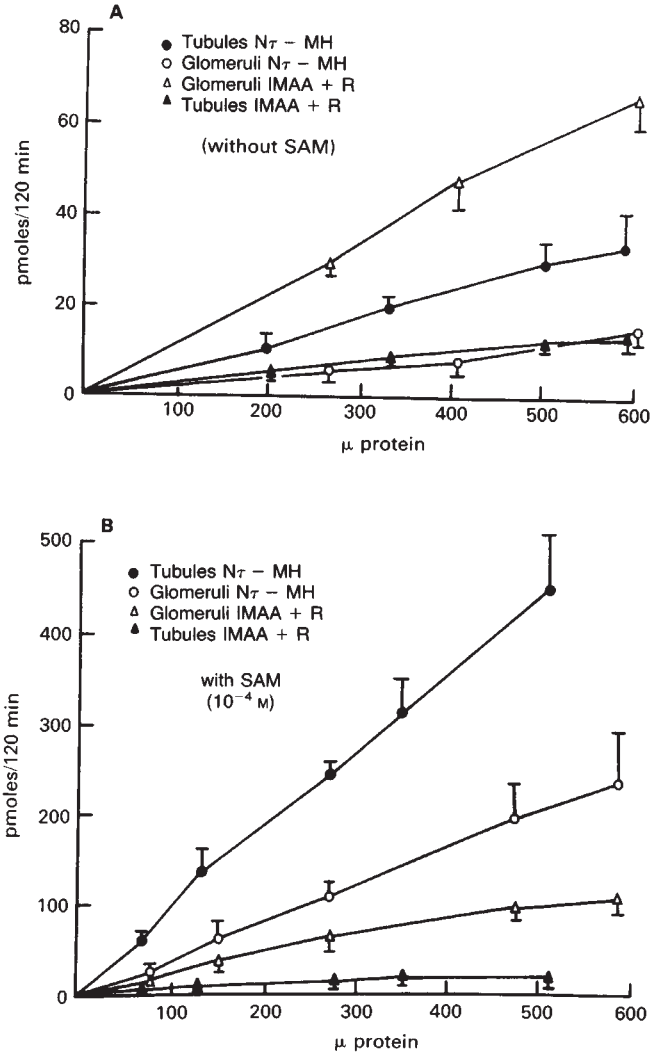

Fig. 3. *The effect of increasing glomerular and tubular protein concentrations on product formation.* Glomeruli and tubules were incubated with 5×10^{-6}M ^{14}C-histamine for 120 min in the absence (**A**) or presence (**B**) of 10^{-4}M S-Adenosylmethionine (SAM). Labelled histamine and metabolites were separated on thin layer chromatography. Note the difference in the scales. Each *point* represents the mean ± SEM of four determinations (two incubation samples aliquoted in duplicates).

10^{-6}M), there was a small decline in the levels of histamine in glomeruli (Δ% − 6.95 ± 4.5 and −6.42 ± 2.6) and in tubules (Δ% − 8.78 ± 4.5 and −7.13 ± 1.9), respectively (Fig. 7). On the other hand, in the presence of SAM, there was a marked decline in the levels of histamine in both glomeruli and tubules. Similar to the experiments with the labelled substrate, the decline in histamine levels was greater in tubules than in glomeruli at both low and high concentrations of histamine added to the incubation (Δ% − 30.54 ± 2.7 and −9.5 ± 2.9 in glomeruli) vs. (Δ% − 57.97 ± 3.9 and 34.37 ± 1.9 in tubules), respectively.

Discussion

These studies demonstrate that cortical tubules have a much higher capacity to degrade histamine than glomeruli and that histamine is differentially catabolized in these two segments of the rat renal cortex. In the absence of exogenous SAM, glomeruli catabolize histamine predominantly via the diamine oxidase pathway while tubules catabolize histamine via the methylation pathway. The predominance of the methylation pathway in tubules over that in glomeruli was observed both in

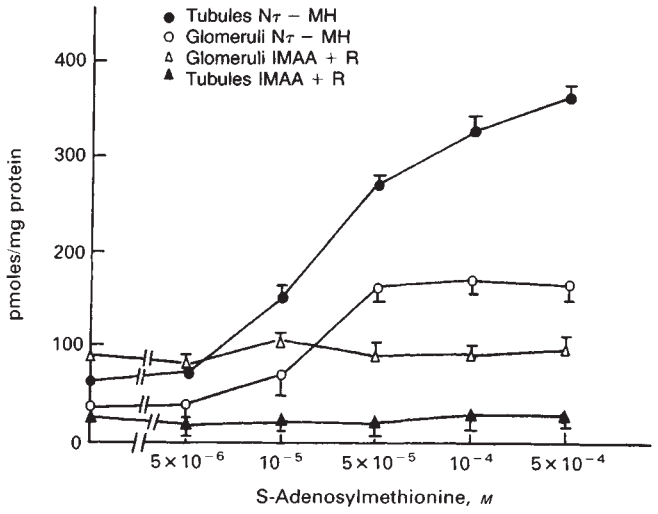

Fig. 4. *The effect of increasing concentrations of S-Adenosylmethionine* (SAM) *on product formation in isolated glomeruli and tubules.* Incubations were carried out in the presence of 5×10^{-6}M ^{14}C-histamine for 120 min. Labelled histamine and metabolites were separated on thin layer chromatography. Each *point* represents the mean ± SEM of four determinations (two incubation samples aliquoted in duplicates).

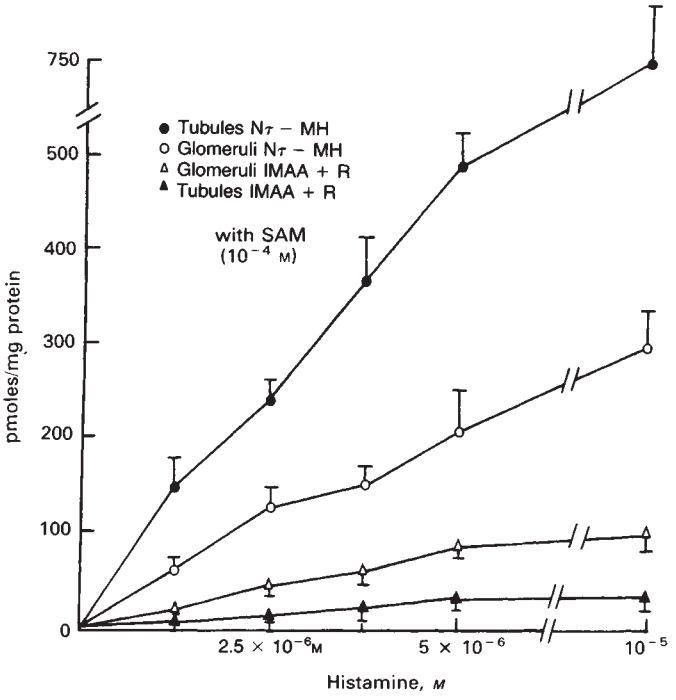

Fig. 5. *Product formation by isolated glomeruli and tubules as a function of increasing concentration of* ^{14}*C-histamine.* Incubations were carried out with varying concentrations of ^{14}C-labelled histamine (by dilution with cold histamine) for 120 min in the presence of 10^{-4}M S-Adenosylmethionine (SAM). Labelled histamine and products were separated on thin layer chromatography. Each *point* represents the mean ± SEM of four determinations (two incubation samples aliquoted in duplicates).

the absence and presence of saturating concentrations of SAM. This observation suggests that the activity of histamine methyltransferase is higher in tubules than in glomeruli. That these differences are not due to differential uptake of radiolabelled histamine or SAM is also supported by the finding that prior permeabilization of the tissue by repeated freezing and thawing did not influence the pattern of distribution of methylation products in either glomeruli or tubules. Although the endogenous content of histamine in glomeruli is higher than that in tubules [10], a change in specific activity of ^{14}C-histamine added to tissues also cannot explain the observed differences in catabolism since the absolute amount of ^{14}C-histamine added exceeds by more than 30-fold the endogenous histamine contents [10]. Furthermore, the studies with nonlabelled histamine show that in the presence of SAM, the rate of decline of histamine levels in tubules was much higher than in glomeruli and thereby confirms the TLC studies.

The higher rate of histamine catabolism in tubules as compared to glomeruli is consistent with the presence of a very active histamine methyltransferase in whole rat kidney [22], which consists predominantly of tubules. The striking, enhancing effect of the methyldonor SAM on the methylation pathway in glomeruli and tubules suggests that its presence may also be a major factor that determines histamine availability in the kidney. A similar requirement of SAM for histamine methylation by whole renal tissue in vitro was observed by other investigators [26, 27]. The concentration of SAM in whole renal tissue in situ is high ($\simeq 5 \times 10^{-5}$M) [28, 29] and, at this concentration of SAM, tubules continue to methylate histamine to a much higher extent than glomeruli. Although extrapolation from in vitro to in vivo studies should be cautious, these experiments carried out in the presence of exogenous SAM are likely to reflect the catabolism of histamine in vivo. Since SAM is a highly unstable compound [29], the amount of SAM remaining in glomeruli and tubules after the isolation procedure is probably negligible and does not reflect in situ levels. It is conceivable that the higher levels of histamine in glomeruli than in tubules that we have observed in our previous studies [10] are not only due to the higher rate of histamine synthesis in glomeruli, as we have demonstrated, but also due to a lower rate of degradation. However, since the distribution of SAM between glomeruli and tubules is not known, the detailed role of additional factors that determine histamine levels in glomeruli and tubules remains to be determined.

The presence of an active diamine oxidase pathway specifically in glomeruli with little activity in the cortical tubular preparation should be briefly considered. The enzyme histaminase is present in the kidney of various species, including the rat [15, 16]. However, since the histamine inhibitor aminoguanidine had no effect on the catabolism of histamine in whole rat kidney slices or renal homogenates in vitro, some investigators concluded that this pathway does not play a role in histamine catabolism in the rat kidney in vitro [26, 27]. It is most likely that such studies reflect primarily effects in tubules, and mask the metabolic activity in glomeruli, which constitute a small percentage of whole renal tissue. Imidazole acetic acid, the major product of this pathway, is a chemoattractant for leukocytes [15] and has recently been shown to inhibit histaminase release from leukocytes [17] and to modulate complement-induced leukocyte function [18]. These effects are specific for imidazole acetic acid but not for other imidazole compounds and have been demonstrated at very low concentrations

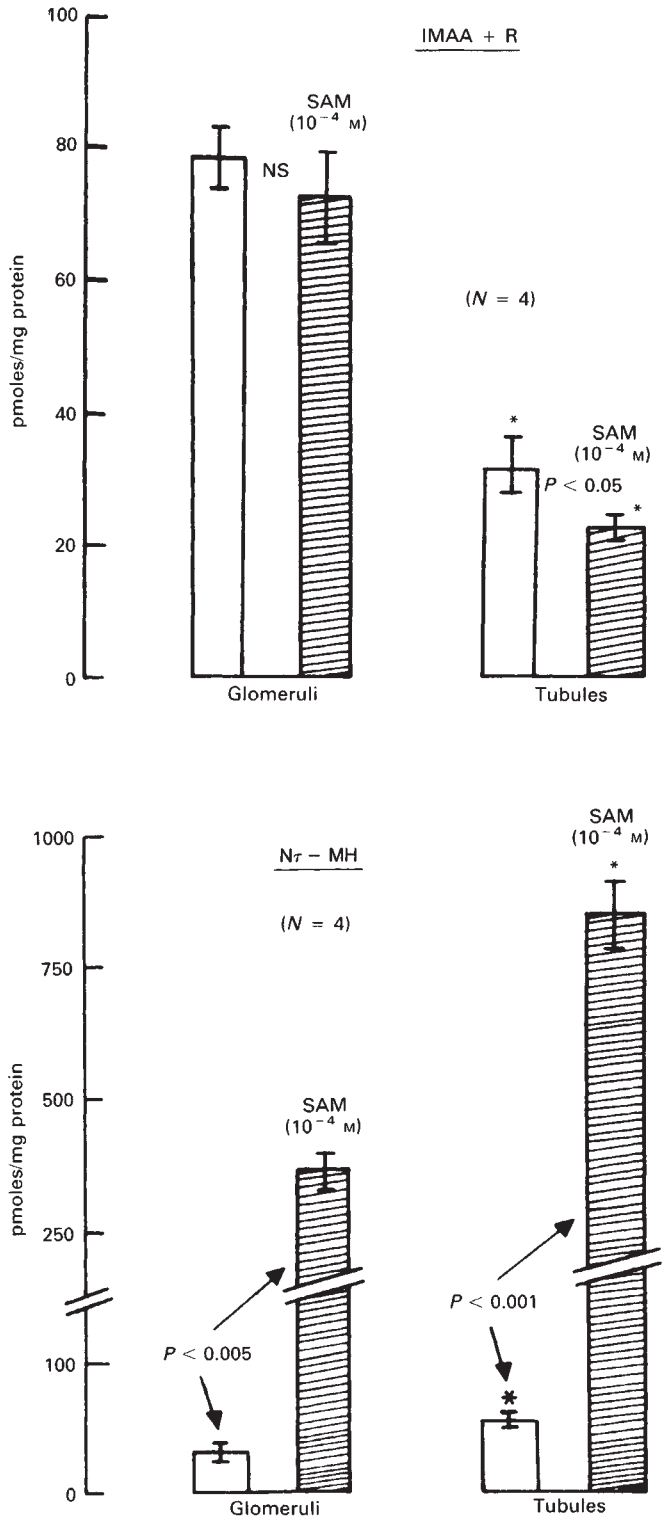

Fig. 6. *Amounts of ^{14}C-labelled imidazole acetic acid and its riboside (IMAA + R)* (upper panel) *and ^{14}C-labelled Nτ-methylhistamine (Nτ - MH)* (lower panel) *formed in glomeruli and tubules incubated with 5 × 10^{-6}M ^{14}C-histamine in the absence* (open bars) *and the presence* (hatched bars) *of 10^{-4}M S-Adenosylmethionine* (SAM) *for 120 min.* Values are mean ± SEM of four experiments (each run in duplicate or triplicate incubation samples). The *asterisk* denotes values in tubules significantly different ($P < 0.05$ or better; unpaired *t* test) from corresponding values in glomeruli.

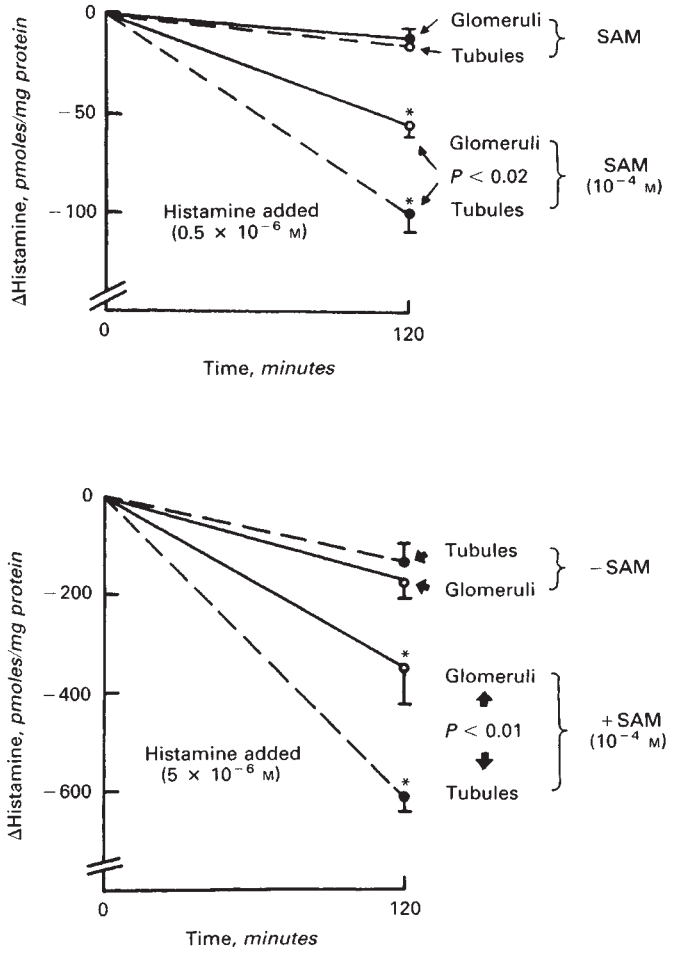

Fig. 7. *Changes in histamine levels in glomeruli* (solid lines) *and in tubules* (broken lines) *after incubations in the absence or presence of 10^{-4}M S-Adenosyl-methionine.* The *upper panel* represents incubations that contained 100 pmoles of nonlabelled histamine (0.5 × 10^{-6}M); lower panel represents incubations that contained 1,000 pmoles of nonlabelled histamine (5 × 10^{-6}M). Histamine levels in control tubes (0 incubation time) and tubes incubated for 120 min were determined by radioenzymatic assay. The *asterisk* denotes values (decrease in histamine) significantly different ($P < 0.05$ or higher degree of significance; paired *t* test) from corresponding levels in the absence of SAM.

(<10^{-9}M) that may be achieved in the kidney. It is conceivable that this histamine product, in addition to histamine itself, may modulate inflammatory responses initiated by either intrinsic glomerular cells or in cells infiltrating the glomerulus in the course of immune-mediated renal injury. Locally produced IMAA may also regulate the rate of histamine destruction and therefore its availability for cells possessing histamine receptors.

The current observations that tubules more actively degrade histamine than glomeruli, that histamine acting via an H_2 receptor exerts a striking, specific stimulatory effect on glomerular cyclic nucleotides production [12, 13], and that glomeruli but not cortical tubules synthesize histamine [10, 11] suggest that glomeruli are major sites of histamine synthesis and action in the kidney while tubules are major sites of histamine degradation. Previous studies have shown that the availability of the substrate-L-histidine and the activity of histidine decarboxyl-

ase, key enzyme of histamine synthesis, may be an important mechanism that regulates histamine levels in various tissues including the kidney [11, 31, 32]. Our studies suggest that, in addition, the activity of the histamine catabolizing enzymes and the availability of their cofactors may play a major role in determining the dynamics of histamine metabolism in various anatomical sites in the kidney. The diamine oxidase inhibitor aminoguanidine and the histamine methyltransferase inhibitor amodiaquine are active not only in vitro but also in vivo [14–16]. In vivo modulation of SAM in the kidney can be achieved by the administration of methionine [28]. Therefore, as demonstrated in other tissues, it should be feasible to modulate the renal and specifically the glomerular content of histamine by either altering its substrate availability, or by changing the activity of its synthesizing or degrading enzymes and their cofactors [31–32]. Such maneuvers may provide a valuable tool for exploring the actions of histamine in physiological or pathological states in the kidney.

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